Report No. IITRI-L6025-5
(Final Report)

EFFECT OF WHOLE-BODY IONIZING IRRADIATION ON ACTIVITY LEVELS OF BLOOD SERUM ENZYMES

March 23, 1965, to May 15, 1967

Contract OCD-PS-64-200
Task Order 37
Work Unit 2531D
IITRI Project L6025

Prepared by

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for

Office of Civil Defense
Office of the Secretary of the Army
through the Technical Management Office
U.S. Naval Radiological Defense Laboratory

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SUMMARY

EFFECT OF WHOLE-BODY IONIZING IRRADIATION ON ACTIVITY LEVELS OF BLOOD SERUM ENZYMES

The purpose of this study was to review the technical literature to obtain information on whether whole-body exposure of animals to ionizing radiation in the approximate LD_{50} dose range for man (300 to 600 rads) induces changes in the activities of specific blood serum enzymes that are dose-related and suitable as a basis for the development of a biological radiation dosimeter. Of the enzyme systems reviewed, lactic dehydrogenase (LDH) isoenzymes and cholinesterase appear to have the most promise.

Values obtained for the ratio of LDH isoenzyme bands 1:2 showed that various levels of ionizing radiation, including the LD_{50} range for man, can be distinguished. At 24 hr after sham irradiation the relative ratio of bands 1:2 of normal (control) male rhesus (Macaca mulatta) monkeys was in the range of 1.0 to 2.5, whereas at 24 hr after 200 to 500 rads of gamma irradiation of monkeys the ratios ranged from 0.88 to 0.36. At radiation levels above 500 rads, the LDH isoenzyme response was further magnified.

The serum cholinesterase activities of various species of animals exposed to whole-body ionizing irradiation generally showed a 15 to 30% decrease, a response that persisted over a 2- to 3-day period. Although this observation was based on a relatively limited number of evaluations, the response was consistent. Additional experimental work, especially with primates, is required for further evaluation of cholinesterase as a biological radiation dosimeter.

The usefulness of a particular enzyme system as an index of radiation exposure is frequently limited by large variations inherent in normal physiological output. Consequently, close integration of the response of a biological indicator with radiation symptomology is necessary to ensure the validity of the biological dose-response measurement. For example, determination and correlation of specific serum enzyme activities and lymphocyte counts may be of prognostic value if performed soon after exposure to whole-body ionizing irradiation.

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EFFECT OF WHOLE-BODY IONIZING IRRADIATION ON ACTIVITY LEVELS OF BLOOD SERUM ENZYMES

I. INTRODUCTION

Significant alteration of serum enzyme activity due to ionizing irradiation is believed to result from the release of cellular enzymes into extracellular fluid. The generally accepted view is that these enzymes diffuse from the cells into the plasma because of increased membrane permeability, which can be considered an early, nonspecific reaction of cells when their metabolism is grossly disturbed.

The purpose of this study was to review the technical literature and obtain information on whether whole-body exposure of animals to various levels of ionizing radiation produces dose-related changes in the activity of specific blood serum enzymes. The ultimate goal is to determine whether these changes provide a basis for the development of a biological dosimeter.

In general, the criteria for a biological dosimeter are:

(1) Specificity of response. Ideally, the response should be induced only by ionizing radiation and not by other physiological disturbances.

(2) Predictability of response. The dosimeter should respond in a mathematically predictable manner to increasing exposures in the range of 200 to 1000 rads.

(3) Time of appearance of response. Evidence of radiation damage should occur soon after exposure, e.g., within 24 hr. Furthermore, the dosimeter should register biological damage over a period of 3 to 6 days.

(4) Simplicity of measurement. The assays should be relatively simple and rapid and preferably should not require highly skilled personnel.

(5) Ready accessibility of test substrate. The test substrate should be readily secured without harm or discomfort to the subject.

II. SOURCES OF INFORMATION

The literature reviewed included:

(1) Nuclear Science Abstracts, 1956-1966

(2) Chemical Abstracts, 1959-1967

(3) Index Medicus, 1963-1966

(4) School of Aerospace Medicine Reports, Brooks Air Force Base, 1960-1966

(5) Radiation Research, 1960-1967

(6) Biological Abstracts, 1965-1966

(7) Science Information Exchange, Abstracts of Projects, 1963-1966

Approximately 315 abstract cards were compiled as reference material. Original articles in domestic and foreign journals were examined to obtain additional information on the response of specific serum enzymes to ionizing radiation.

III. TECHNICAL REVIEW

The serum enzymes covered in this review were:

(1) Aldolase

(2) Acid and alkaline phosphatases

(3) Carbonic anhydrase

(4) Catalase

(5) Cholinesterase

(6) beta-Glucuronidase

(7) Glutamic oxalacetic transaminase

(8) Lactic dehydrogenase and lactic dehydrogenase isoenzymes

Although considerable definitive information is available regarding the effects of ionizing radiation on serum enzyme activity, conflicting data and conclusions are prevalent because of the different types of animals and various dose rates used by individual investigators. The information presented in this report is a representative sampling of all the data evaluated during this study. Particular emphasis has been placed on enzymes that appeared to offer promise as biological dosimeters.

A. Lactic Dehydrogenase and Lactic Dehydrogenase Isoenzymes

1. Lactic Dehydrogenase

Kärcher (ref. 1) subjected rats to whole-body exposures of 800-rads of x-irradiation and found that serum lactic dehydrogenase (LDH) activity rose to a peak 6 hr after irradiation, declined to normal in 24 hr, reached a peak again in 3 to 4 days, and then declined steadily to a low point on the seventh day (Figure 1).

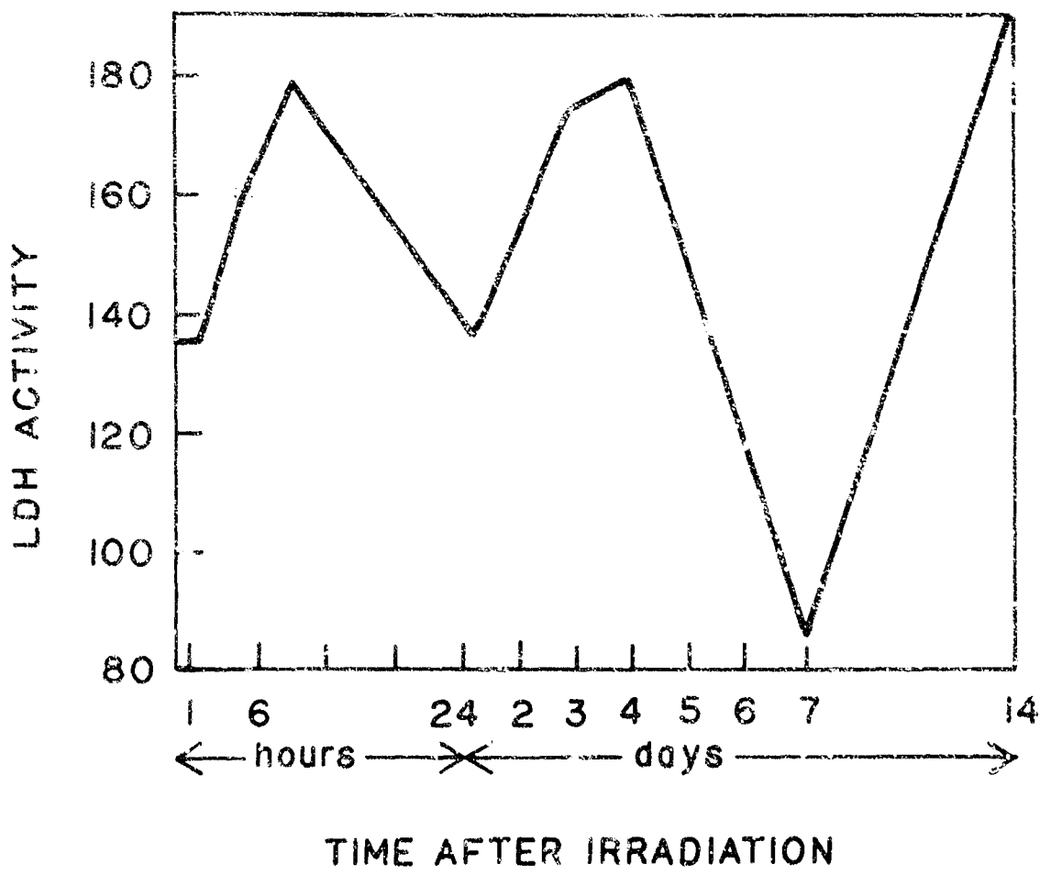

Figure 1

EFFECT OF 800 RAD X-IRRADIATION ON LDH ACTIVITY
OF WHOLE-BODY IRRADIATED RATS
(ref. 1)

In a subsequent study (ref. 2) he exposed rats to 800 rads of x-irradiation and reported that serum LDH activity rose 2-fold during the first day, from a level of 13 activity units to a maximum of 26 units, and then declined to normal by the fifth day.

Volek (ref. 3) followed changes in the serum LDH activity of rats from 4 hr to 4 weeks after whole-body x-irradiations of 25, 100, 600, and 1000 rads. Serum LDH activity fell 4 hr after irradiation, except for the 1000-rad dose, which produced no effect at this time. The 600-rad dose diminished activity 35%. After 24 hr, the activity was elevated approximately 20% by the 100-, 600-, and 1000-rad doses. Thereafter, activity tended to decline. After 3 days, the LDH activity of the 600-rad irradiated rats was about 50% of that of normal rats (Figure 2).

Hornung et al (ref. 4) exposed male white rats to 600- ads of x-irradiation and observed that serum LDH activity decre sed approximately 42% 2 days after irradiation and returned to about normal in 4 days. Thereafter, the activity declined to about 50% of normal by 10 days after irradiation (Figure 3).

Working with rabbits, Albaum (ref. 5) found that whole-body exposure to 750 rads of x-ray radiation produced an increase in serum LDH activity within 6 hr, which was sustained over the 24-hr period measured. However, as noted in Table 1, the control animals showed a higher serum LDH activity in 6 hr than the irradiated animals. By 24 hr the LDH activity of the control animals returned to normal, but the irradiated animals continued

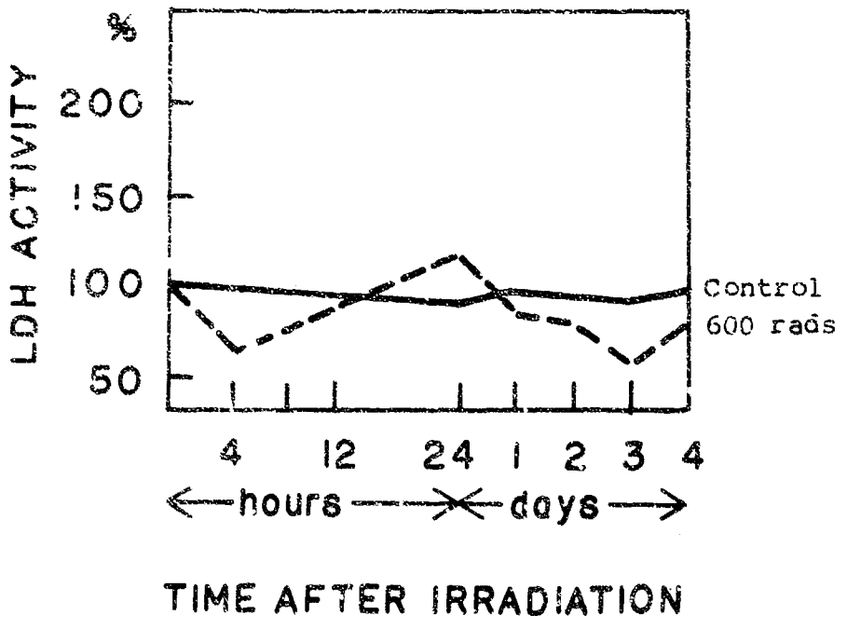

Figure 2

EFFECT OF 600 RAD X-IRRADIATION ON LDH ACTIVITY
OF WHOLE-BODY IRRADIATED RATS
(ref. 3)

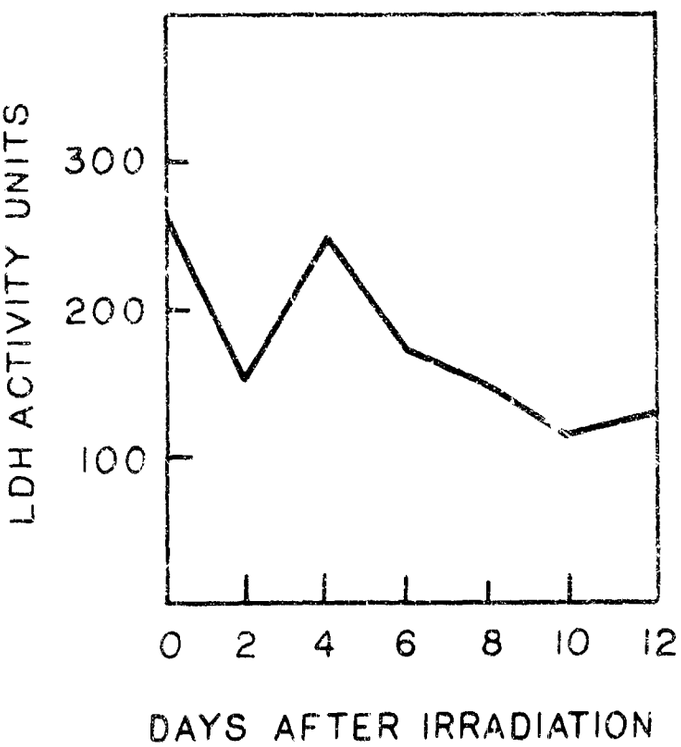

Figure 3

EFFECT OF 600 RAD X-IRRADIATION ON LDH ACTIVITY OF RATS
(ref. 4)

to show elevated activity. The increase in activity of the controls can be attributed to handling of the animals; the stress presumably promotes increased serum enzyme activity.

Table 1

LACTIC DEHYDROGENASE ACTIVITY IN CONTROL AND IRRADIATED RABBITS
(750-rad Whole-Body Irradiation)

	0 hr	6 hr		24 hr	
Sample	Activity	Activity	% Change	Activity	% Change
Control	421	935	+122	500	+18
Irradiated	496	830	+ 67	820	+65

Work on LDH activity of primates, Macaca mulatta, exposed to various levels of 2 MEV of whole-body x-irradiation was performed by Dalrymple et al (ref. 6). As shown in Table 2, serum LDH values increased significantly during the first 2 days after irradiation and then started to decrease. They concluded that the serum LDH level is a reasonably good indicator of radiation injury, if it is measured during the first 2 days after irradiation and if the subject receives doses in the range of the $LD_{50/30}$. The degree of elevation of enzyme activity could not, however, be accurately correlated with ultimate survival or with dose for a given animal.

Table 2

LACTIC DEHYDROGENASE ACTIVITY IN CONTROL AND IRRADIATED RABBITS
(2 MEV of X-Irradiation)

Radiation Level, rads	0 hr	24 hr		48 hr		96 hr	
	Activity	Activity	% Change	Activity	% Change	Activity	% Change[a]
0	333	470	+41	562	+69	466	+40
360	358	833	+122	829	+131	452	+36
446	467	1170	+150	783	+68	497	+49
538	322	819	+154	1237	+284	649	+95
624	363	1411	+298	997	+174	794	+138
716	430	1342	+212	1228	+186	706	+112

[a]Compared with controls.

The literature evaluated indicates that although changes in serum LDH activity are a consequence of irradiation, the variability of the dose response precludes consideration of the enzyme as a quantitative biological dosimeter.

2. LDH Isoenzymes

The term "isoenzyme" is used to describe electrophoretically distinguishable enzymes with similar substrate specificities. The normal serum total isoenzyme pattern reflects all the tissue-specific isoenzyme patterns. Damage to a tissue alters the normal serum isoenzyme pattern and produces an LDH isoenzyme pattern indicative of the tissue pathology. The pattern remains abnormal until the tissue has reestablished its normal function.

Although only minor changes in total serum LDH activity may be effected by radiation exposure, significant changes may occur in tissue metabolism. Such tissue respiration changes are reflected in serum LDH isoenzyme patterns that demonstrate a statistically significant change in the aerobic/anaerobic (H/M) isoenzyme ratio as a function of radiation exposure. Thus, an immediate rise in total LDH activity is manifested by a marked increase in the anaerobic LDH fractions represented by bands 4 and 5.

Hornung et al (ref. 4) studied changes in the LDH isoenzyme patterns of the serum of male rats immediately after exposure to 600 rads of whole-body Co^{60} irradiation. The activity changes are shown in Table 3.

Table 3

EFFECT OF X-IRRADIATION ON SERUM LDH ISOENZYMES
(600 rads)

Isoenzyme Band	LDH Fraction, %		
	Control	Irradiated	% Change
1	34.1	15.2	-55.5
2	26.5	16.7	-37
3	6.6	5.7	-13.5
4	1.8	12.4	+59.0
5	31.0	50.0	+61.4

The anerobic bands, 4 and 5, significantly increased after irradiation. This increase appears to be related to the fractions that decreased in most of the organs after irradiation. Märcher (ref. 7) points out that the time course of total LDH activities in serum and organ homogenates cannot provide information about the radiation sensitivity of individual organs after a single whole-body irradiation. However, the isoenzyme patterns of these organs can provide valuable hints.

Hawrylewicz and Blair (ref. 8,9) conducted studies to determine whether serum LDH isoenzyme response in male rhesus monkeys, Macaca mulatta, subjected to ionizing radiation is unique and is a function of dose. The doses studied were 200, 300, and 500 rads of gamma radiation at a dose rate of 20 rads/min. With normal control monkeys, at 24 hr after sham irradiation, the relative ratio of isoenzyme bands 1:2 was in the range of 1.0 to 2.5, whereas with irradiated monkeys the ratio ranged from 0.88 to 0.36.

As shown in Figure 4, 24 hr after exposure to 200 rads, the ratio of isoenzyme bands 1:2 dropped from 1.40 to 0.45 and remained abnormal over the 216-hr test period. Figure 5 shows that 24 hr after irradiation with 300 and 500 rads, the ratios dropped from 1.53 to 0.88 and from 1.77 to 0.83, respectively. By 72 hr recovery of the ratio to 1.44 for the 300-rad group and to 1.32 for the 500-rad group was observed. By 168 hr the ratios reached peak values of 4.25 in the 300-rad group and 3.90 in the 500-rad group, increases of approximately 70 and 55%, respectively over the control value. At levels above 500 rads the serum LDH isoenzyme response was further magnified and could be readily distinguished from responses to less irradiation. The overall changes described here preceded the usual hematopoietic response, shown in Figure 6, which is not considered to be a reliable radiation dose response indicator until 48 hr after irradiation.

The authors suggest that the increase in aerobic LDH components (H-LDH isoenzymes) is a response to the increased utilization of energy during recovery and that the "crossover point" is a manifestation of decreasing cell death and increasing cellular enzyme activity. These alterations appear to reflect altered metabolic activities observed in the heart, testes, and pancreas. The authors further postulate that serum LDH isoenzyme determinations are reliable indicators of early reaction to subacute irradiation and that they add much supplemental value to the use of the white blood cell count as a biological radiation dosimeter. Since the isoenzyme assay time has been reduced to approximately 45 min (ref. 10) and since doses of

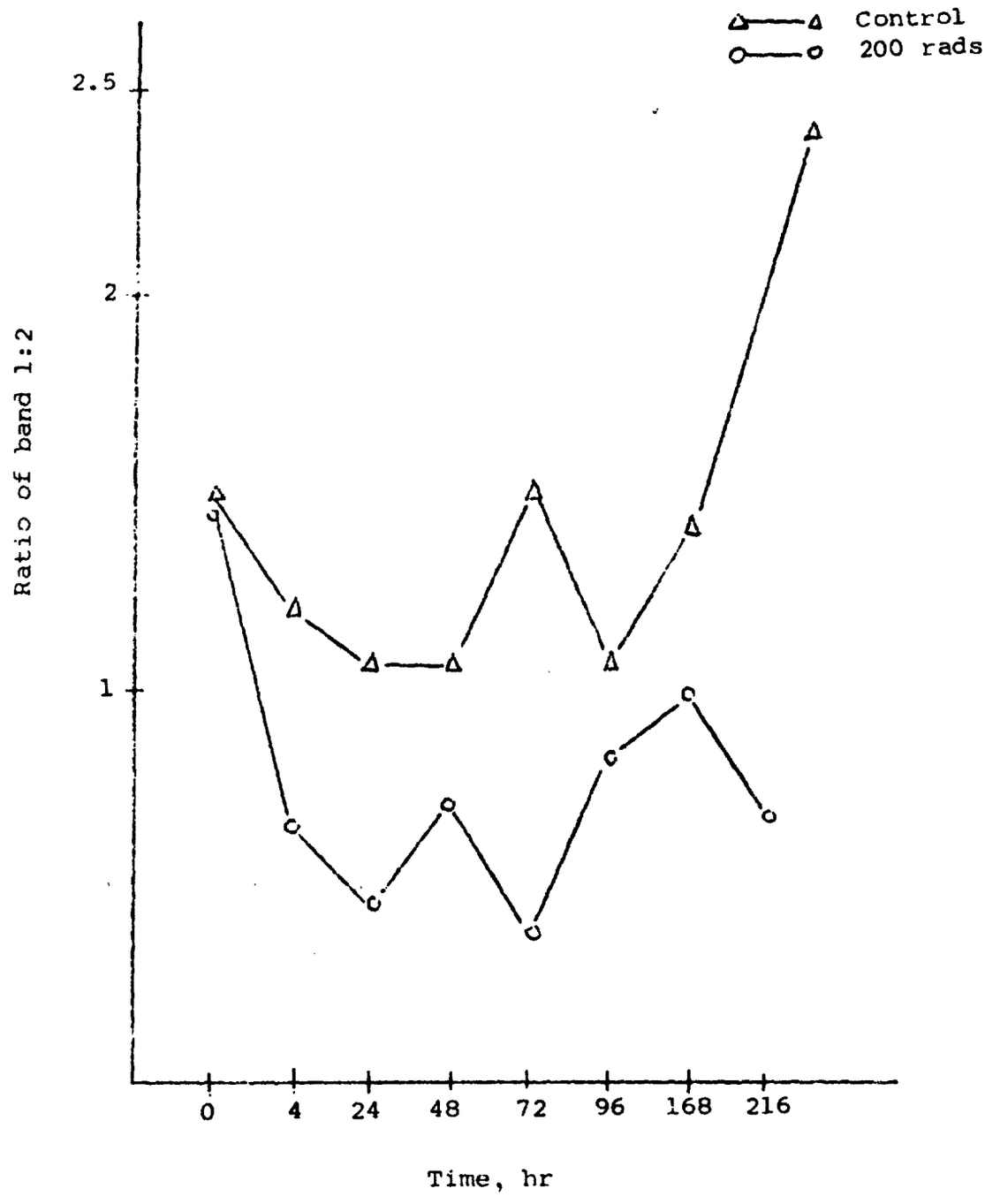

Figure 4

LDH ISOENZYME BAND 1:2 RATIOS FOR MONKEYS IRRADIATED WITH 200 RADS OF GAMMA RADIATION (ref. 8,9)

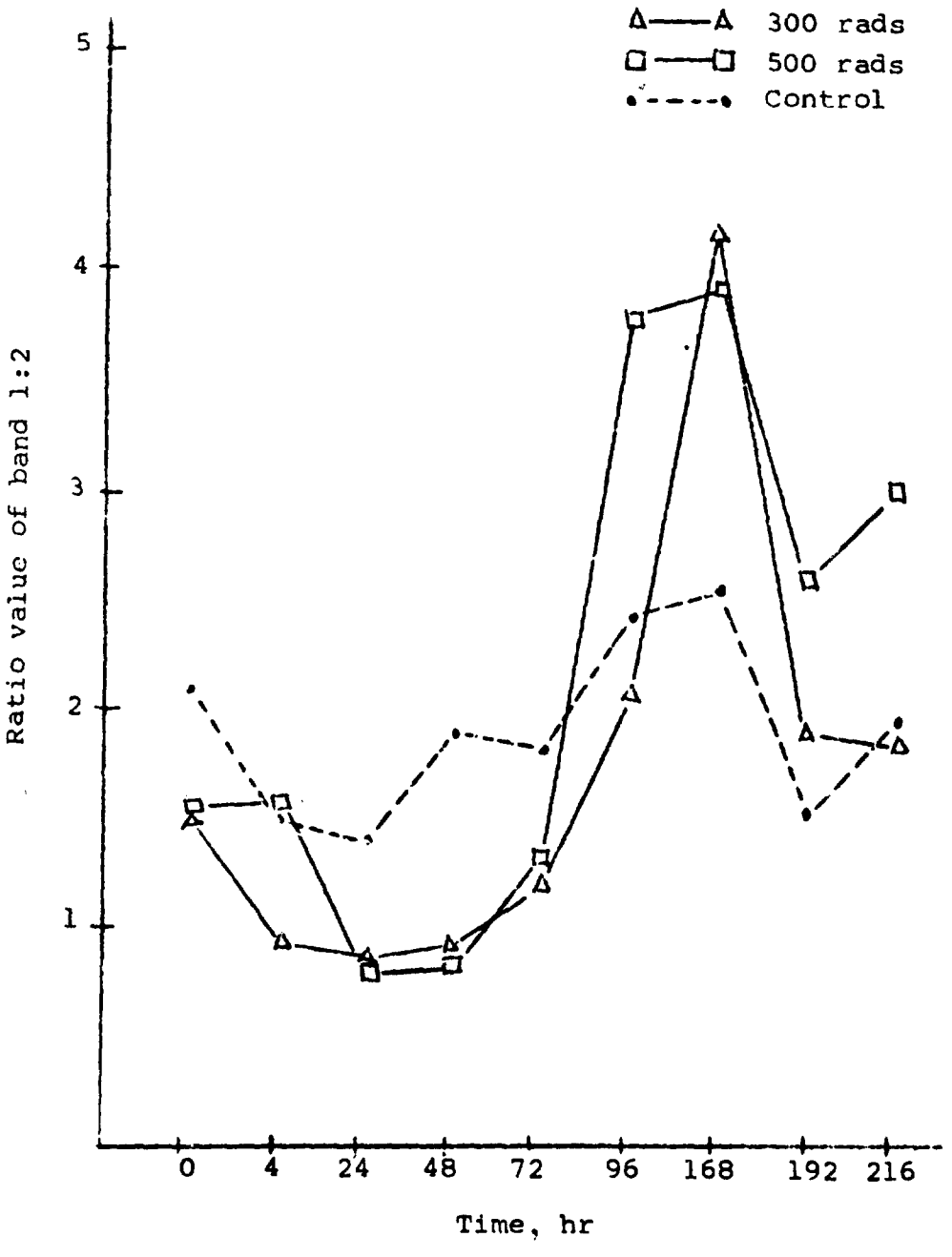

Figure 5

LDH ISOENZYME BAND 1:2 RATIOS FOR MONKEYS
IRRADIATED WITH 300 and 500 RADS OF GAMMA RADIATION
(ref. 8,9)

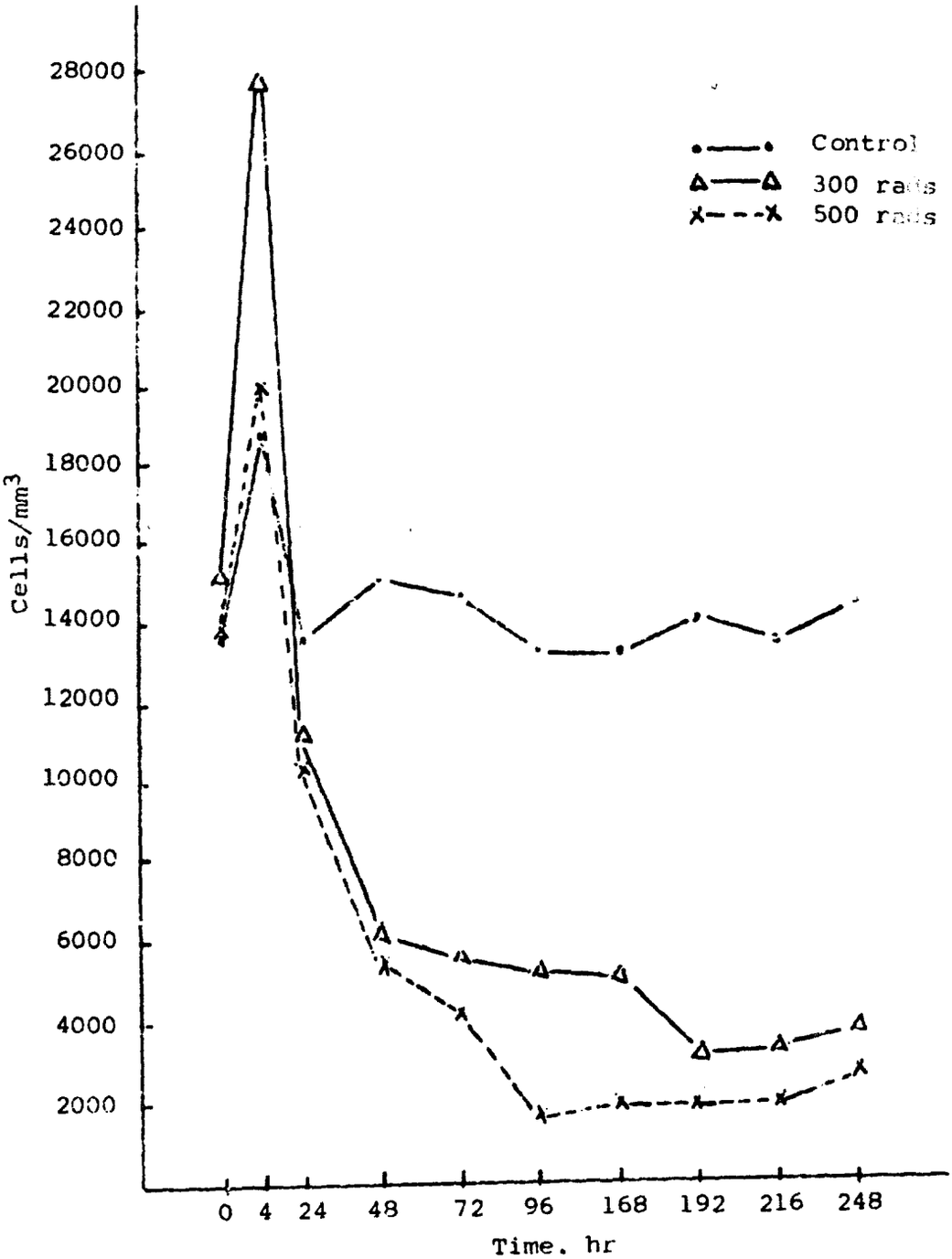

Figure 6

TOTAL WHITE BLOOD CELL COUNT OF IRRADIATED MONKEYS
(ref. 8,9)

500, 300 and 200 rads can be distinguished, isoenzyme patterns appear to warrant further evaluation as a biological radiation dosimeter.

B. Cholinesterase

Serum cholinesterase activity of animals exposed to whole-body ionizing irradiation generally decreases, and the effect persists for the 3-day period desired for a biological radiation dosimeter. This response has been observed to various degrees in a number of animal species.

Lundin (ref. 11) exposed guinea pigs to 400 rads of whole-body x-irradiation and found that cholineserase activity decreased approximately 30% during the first two days after irradiation (Figure 7). The response confirmed results previously reported by Ord and Stocken (ref. 12), who used guinea pigs, and by Luthy (ref. 13), who used mice.

Zubkova and Chernavskaya (ref. 14) irradiated rats with 1000 rads of x-rays and measured serum cholinesterase activity at various time intervals thereafter. At this radiation dose level, the approximate loss in activity was 16% within 5 to 45 min after irradiation. However, at 3 days the activity in the serum rose sharply (Table 4).

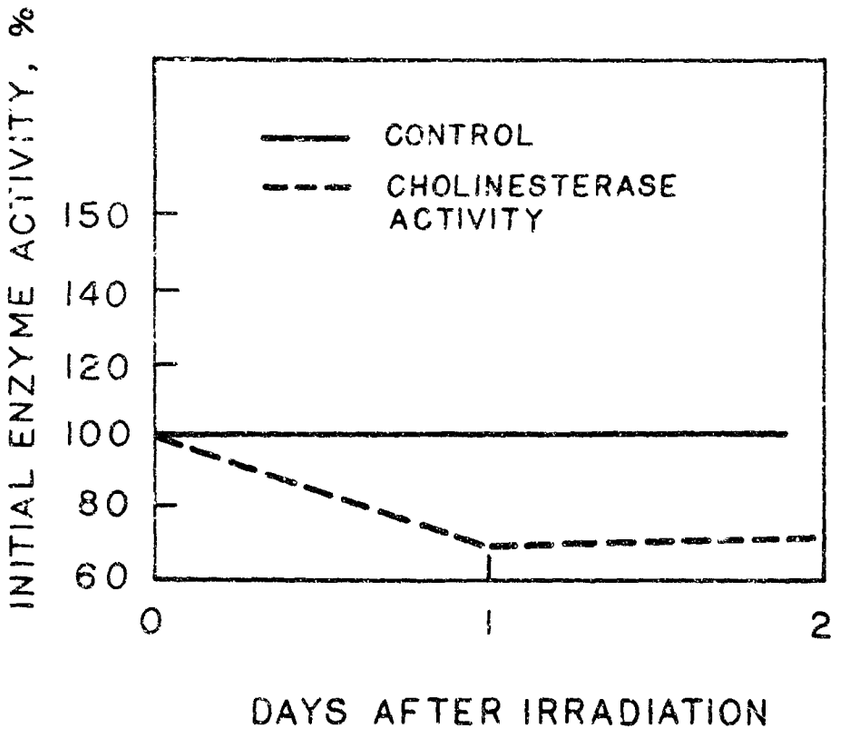

Figure 7

EFFECT OF 400 RAD X-IRRADIATION
ON SERUM CHOLINESTERASE ACTIVITY OF GUINEA PIGS
(ref. 11)

Table 4

CHANGES IN SERUM CHOLINESTERASE ACTIVITY AFTER IRRADIATION
(1000 rads of x-rays)

Time after Irradiation	Cholinesterase Activity Units
Control	71.3
5 min	63.0
45 min	60.0
3 days	85.0

Doull and Cummings (ref. 15) subjected male and female rats to 800 rads of x-irradiation and assayed their serum for cholinesterase activity 3 days after irradiation. Acetyl-β-methylcholine chloride was used as a substrate to measure true (specific) cholinesterase activity, and acetylcholine chloride and benzoylcholine chloride were used to measure pseudo (nonspecific) cholinesterase activity. The true cholinesterase activity of female rat serum was unchanged, but the pseudo enzyme activity was markedly reduced. Thus, 3 days after irradiation, the pseudo cholinesterase activity of female rat serum was decreased to approximately 70% of the control when acetylcholine chloride was used as the substrate, and to 44% of the control when benzoylcholine chloride was used as the substrate. The cholinesterase activity of the serum of male rats was not affected by 800 rads of x-irradiation. Table 5 shows the results obtained with female rats.

Table 5

EFFECT OF 800 RADS OF X-RAY ON THE CHOLINESTERASE ACTIVITY OF THE SERUM OF FEMALE RATS
(substrate = acetylcholine chloride)

Before Irradiation	After Irradiation			
	1st Day	2nd Day	3rd Day	4th Day
15.3	14.5			
12.6	11.2			
18.3	14.9			
12.3		9.2		
19.5		13.8		
14.2		11.5		
15.9			10.2	
15.5			10.6	
14.6			11.2	
14.4				9.8
16.8				10.4
14.3				9.1
Average 15.3	13.6	11.5	10.7	9.8
Percent of control activity	88%	85%	70%	64%

Williams et al (ref. 16) exposed male rats to gamma radiation from a Co^{60} source and found that whole blood cholinesterase activity was depressed approximately 17%, particularly in the 3- to 10-day period after irradiation. As shown in Table 6 and Figure 8, cholinesterase activity, measured as the difference between initial and final pH (ΔpH), decreased with increasing radiation dose. The dose response curve was based upon whole blood cholinesterase activity on the 10th day after gamma-irradiation. Peak significant depression of whole blood cholinesterase activity occurred on the 10th day after irradiation.

Table 6

EFFECT OF GAMMA RADIATION DOSE
ON BLOOD CHOLINESTERASE ACTIVITY IN RATS
(10 days after irradiation)

Radiation Dose, rads	Δ pH
0	1.13
75	0.97
150	0.89
300	0.86
600	0.82

Tominiz (ref. 17) irradiated male rats with 600 rads of gamma radiation and reported that cholinesterase activity dropped 25% in 3 hr. At the end of 24 hr, the activity was still 20% less than that of controls.

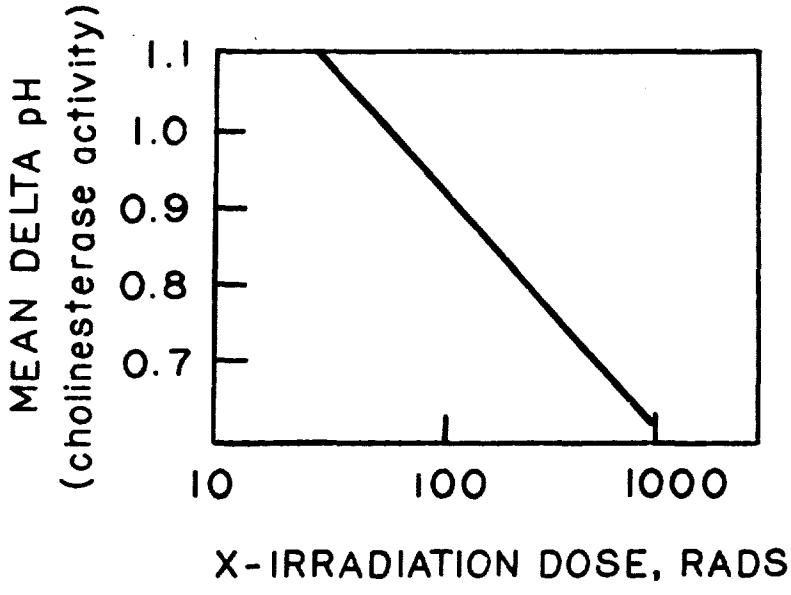

Figure 8

EFFECT OF RADIATION DOSE ON CHOLINESTERASE ACTIVITY
OF RAT WHOLE BLOOD
(ref. 16)

Davydov (ref. 18) studied cholinesterase activity in blood serum of dogs irradiated with 400 rads of gamma radiation. Cholinesterase activity in the blood stream was decreased approximately 64 $\pm$ 6.3% 4 hr after irradiation.

Sabine (ref. 19) irradiated young adult female mice with 25 to 300 rads of whole-body x-irradiation and observed that the cholinesterase activity of the erythrocytes increased. For mice receiving 300 rads, high values were found 3 to 5 days after irradiation. By the end of the first week there was a sharp fall in titer values far below normal. Recovery began during the second week and was apparently complete by the end of the third week. These results are illustrated in Figure 9. Cholinesterase titers were expressed as per unit volume of cells. Each control mean was set equal to 1.0, and experimental values were expressed as decimal fractions of the control value.

French and Wall (ref. 20) exposed rhesus monkeys to 800 rads of whole-body x-irradiation and measured cholinesterase activity in isolated intestinal loops. Their results are shown in Figure 10. There was a small decrease in activity during the days immediately following irradiation, a marked increase above the control during days 4 to 6, and a decrease to below normal during days 6 to 10.

In summary, whole-body ionizing irradiation of guinea pigs, dogs, and rats generally appears to cause a significant decrease in serum cholinesterase activity that persists for 3 days after irradiation. On the other hand, a study that employed rhesus monkeys as the experimental animal (ref. 20) showed only a small decrease in enzyme activity within 2 days after irradiation and a

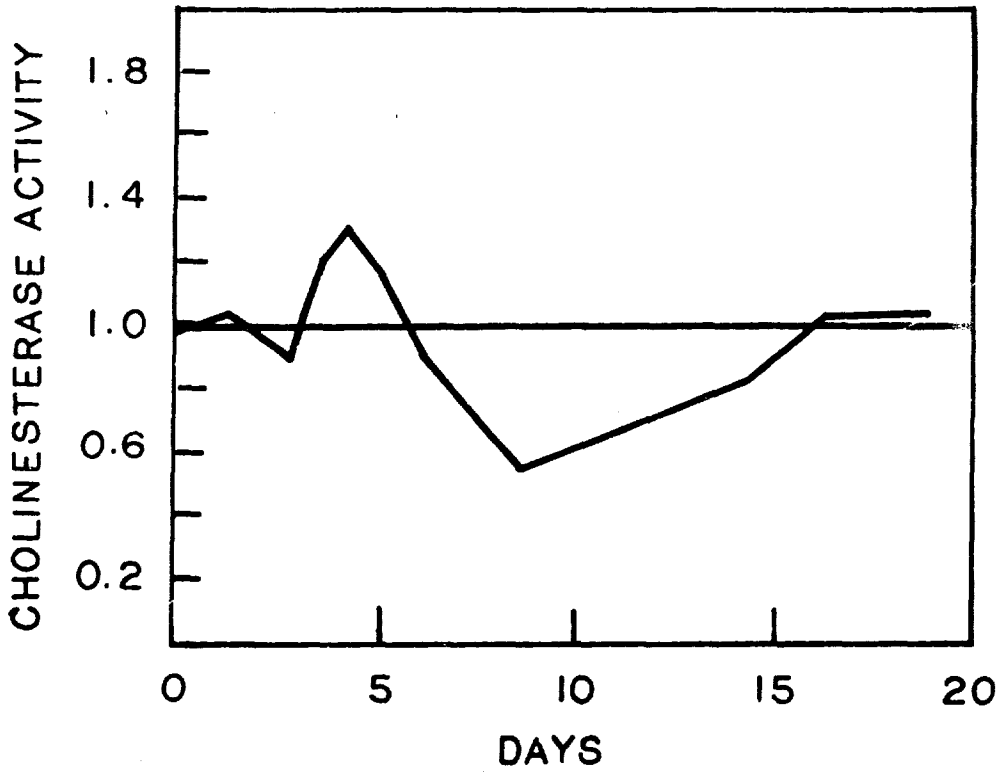

Figure 9

EFFECT OF 30; RAD X-IRRADIATION ON CHOLINESTERASE ACTIVITY
OF MICE ERYTHROCYTES
(ref. 19)

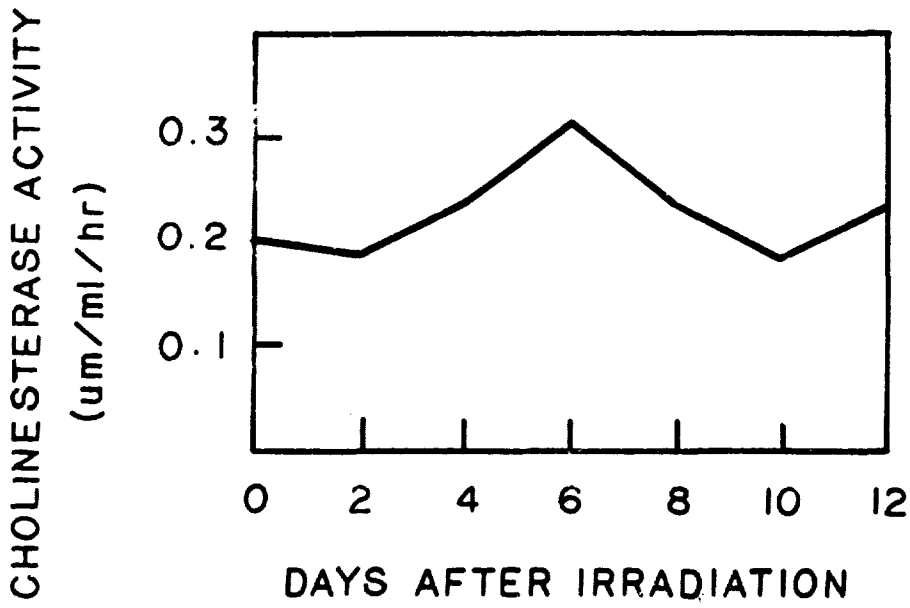

Figure 10

EFFECT OF 800 RAD X-IRRADIATION ON CHOLINESTERASE ACTIVITY OF RHESUS MONKEYS
(ref. 20)

relatively large increase in activity during the 2- to 6-day period. Despite the need for further studies with monkeys, the significant decreases in serum cholinesterase activity reported for irradiated animals suggests that cholinesterase activity warrants further evaluation as a biological radiation dosimeter.

C. Catalase

A number of reports in the literature indicated a drop in catalase activity after whole-body exposure of animals to ionizing radiation.

Matsumoto (ref. 21) irradiated female rats with 300 to 600 rads of Co^{60} irradiation. As shown in Figure 11, catalase activity showed the lowest values 6 hr after irradiation; the activity increased gradually thereafter. With 1200 rads, catalase activity decreased rapidly and did not increase thereafter.

Jonderko (ref. 22) irradiated rabbits with 500, 700 and 1000 rads of x-irradiation and observed that catalase activity decreased during the first 24 to 48 hr after irradiation (Figure 12). No correlation was found between the decrease in activity and radiation dose. Jonderko concluded that the determination of catalase activity for the evaluation of post-irradiation effect is of value only when employed immediately after irradiation.

Darakhvelidze (ref. 23) irradiated rabbits with 400, 600, and 1200 rads of x-irradiation. Catalase activity in the blood decreased independently of the irradiation dose. However, the author postulated that determination of catalase activity and examination of white blood cells may be of prognostic value if performed soon after irradiation. Tuinov (ref. 24) observed a

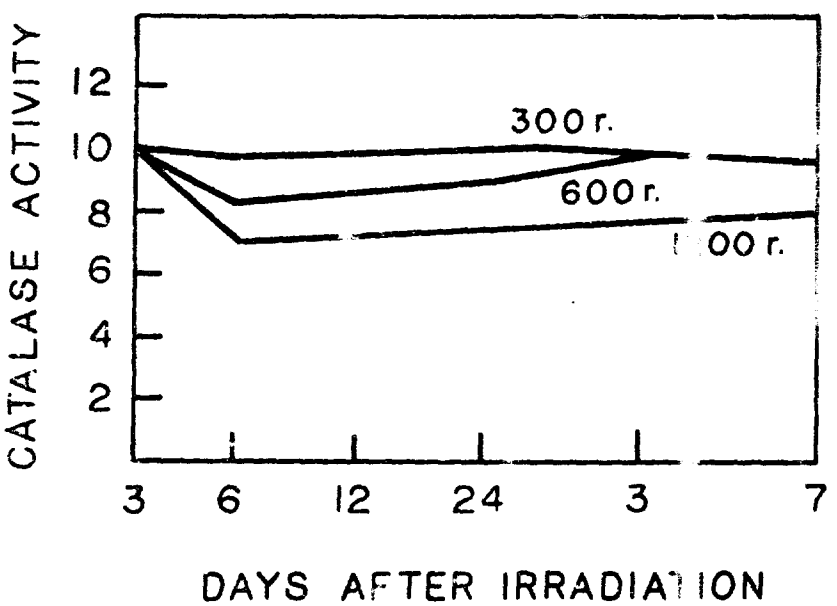

Figure 11

SERUM CATALASE ACTIVITY OF RATS AFTER WHOLE-BODY Co^{60} IRRADIATION (ref. 21)

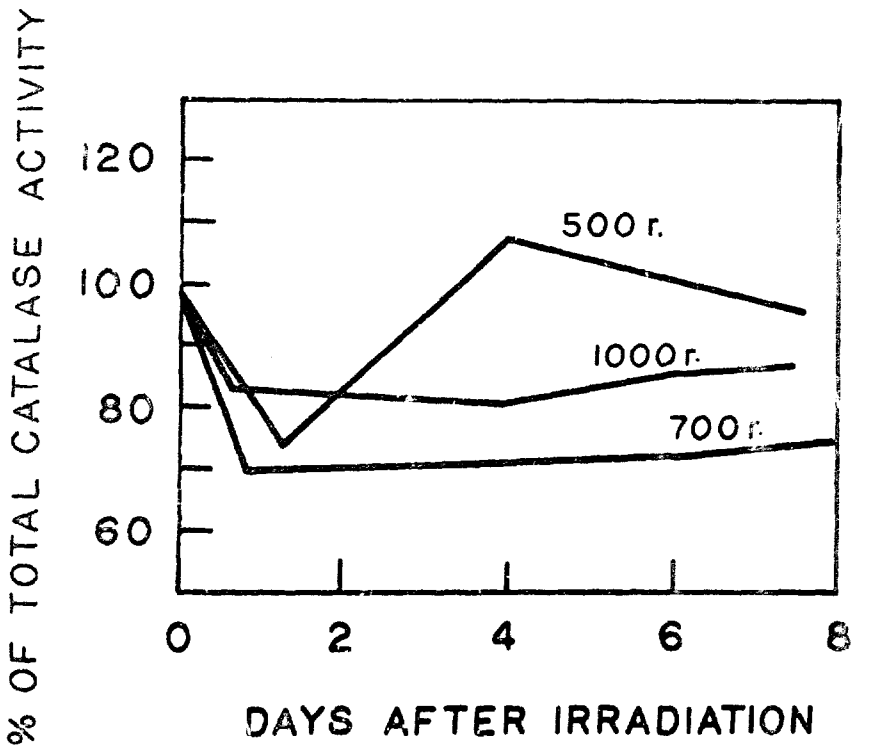

Figure 12

SERUM CATALASE ACTIVITY OF RABBITS AFTER GAMMA-IRRADIATION
(ref. 22)

drop in the catalase index of blood 5 min after exposure to 600 and 650 rads of x-irradiation; the drop continued during the next 20 to 30 min. The maximum drop in enzyme activity was observed during the terminal stages when the catalase activity decreased to about one-third of normal values.

Although serum catalase activity generally decreased significantly with whole-body irradiation, its use as a biological radiation dosimeter appears limited because the decreases apparently were not dose dependent. More work, particularly with primates, is required before definite conclusions can be made.

D. Glutamic Oxalacetic Transaminase (GOT)

Varied SGOT responses to whole-body irradiation of animals have been reported.

Becker (ref. 25) exposed male rats to x-irradiation and reported elevated SGOT levels, as shown in Table 7. The greatest average elevation (expressed as units above preirradiation values and corrected for control variations) was demonstrated by the 900-rad group (460 units at 9 hr); the maximal values for the 300- and 600-rad groups, at 6 hr, were slightly lower. By 24 hr, the enzyme elevations were no longer significant. There was also considerable overlap of values for the individual animals receiving different total doses, as well as considerable variation with groups of animals receiving the same total dose. Becker concluded that although various radiation doses produce differences in the amplitude of enzyme activity, no dosimetric application was indicated.

Table 7

EFFECT OF IRRADIATION DOSE ON SGOT LEVELS IN RATS

Total Dose, rads	SGOT Units of Activity; Dose Rate, 298 rads/min
0	15
150	25
300	350
450	290
600	395
750	310
900	460
1050	20

Oswald (ref. 26) reported that a single dose of 1000 rads of x-irradiation of rats did not significantly alter SGOT activity. His results are shown in Table 8.

Table 8

EFFECT OF RADIATION ON SGOT LEVELS IN RATS
(1000 rads of x-irradiation; single dose)

Time after Irradiation	SGOT Activity Units
0	154.1
3 hr	168.6
1 day	201.9
2 day	164.6
3 day	163.3

Braun (ref. 27) subjected rats to 600 rads of whole-body x-irradiation and measured SGOT activity 2 to 12 days later. As shown in Figure 13, SGOT activity decreased after irradiation.

Milch and Albaum (ref. 28) found that rabbit SGOT activity increased after whole-body exposure to 500, 750, and 1000 rads of x-irradiation. The 500-rad dose produced an elevation over the sham controls at 6 and 24 hr but not at 3 hr. The 750- and 1000-rad doses brought about marked alterations at 3, 6, and 24 hr. There appeared to be no statistically significant difference between the last two dose levels. Their results are given in Table 9.

Table 9

SGOT ACTIVITY IN WHOLE-BODY X-IRRADIATED RABBITS

Treatment	Activity Units Time after Irradiation, hr 0	3	6	24
Sham control	22	35	30	24
500 rads	24	33	38	31
750 rads	22	65	50	45
1000 rads	26	52	66	42

Kessler (ref. 29), also working with rabbits, reported that increased SGOT activity occurred within 5 hr after whole-body x-irradiation (200 to 1000 rads) and that the level remained elevated for 25 hr. After 49 hr, SGOT levels returned to normal. There appeared to be no correlation between SGOT activity and radiation dose.

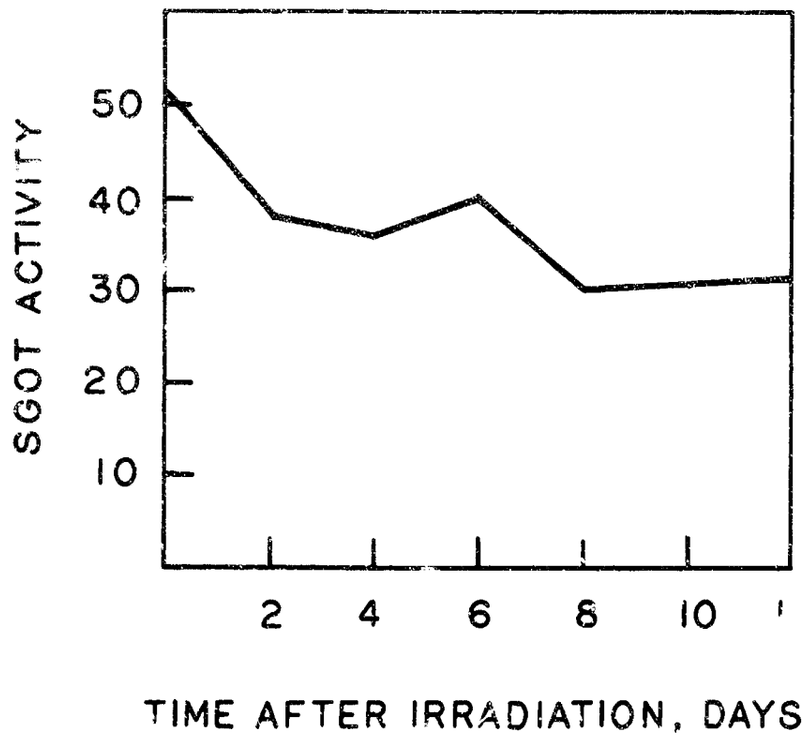

Figure 13

EFFECT OF 600 RAD X-IRRADIATION ON
SERUM GLUTAMIC OXALACETIC TRANSAMINASE ACTIVITY OF RATS
(ref. 27)

Other investigators have also reported observations on SGOT activity after whole-body irradiation. These observations are summarized in Table 10. In general, it appears that SGOT increases markedly in irradiated rats within 6 hr after irradiation, but the activity returns to approximately normal in 24 hr. Rabbits apparently are more susceptible than rats since increased SGOT levels are maintained longer.

E. Alkaline and Acid Phosphatases

Varied responses of serum phosphatase activity to whole-body x-irradiation of animals were noted in the literature.

Dimitrow (ref. 36) exposed dogs to 1250 rads of x-irradiation in a single dose and found that serum alkaline phosphatase activity increased 100% in 3 days. By the 4th day the values had increased 500% over normal values. Dalrymple (ref. 37) studied the effect of 2 MEV of x-irradiation on the alkaline phosphatase activity of irradiated primates. His results, shown in Table 11, indicate that the response was variable and not dose dependent.

Sviderskaya (ref. 38) studied the activity of alkaline phosphatase of the blood in guinea pigs irradiated with 450 to 500 rads of irradiation. Phosphatase activity decreased by 60% 6 days after irradiation; the maximum drop was observed 10 to 15 days after irradiation (activity was 8 to 13% of the initial value).

Chobanova (ref. 39) determined serum alkaline phosphatase activity in dogs irradiated with 440, 800 and 1250 rads. Phosphatase activity values increased but showed no quantitative correlation with the dose applied. Sahasrabudhe (ref. 40) exposed rats to 600 rads of whole-body x-irradiation and found that the levels

Table 10

EXPERIMENTAL OBSERVATIONS ON SGOT ACTIVITY OF IRRADIATED ANIMALS

Investigator	Animal	Dosage	Observations
Brent (ref. 30)	Rats, rabbits	700 rads of x-irradiation	A small but significant rise in SGOT in the rabbits 24 hr after irradiation; no significant alteration in the rats.
Nasek and Sevala (ref. 31)	Rabbits, dogs	600-800 rads of x-irradiation	A single dose of 600 rads produced a marked increase in SGOT activity of rabbits; dogs required a dose of 800 rads.
Andri (ref. 32)	Rats	600 rads of x-irradiation	Increased SGOT activity 3 hr after irradiation; returned to normal after 24 hr.
Almonte (ref. 33)	Mice	100 rads of x-irradiation	SGOT activity increased approximately 40% 6 hr after irradiation; the response did not appear to vary linearly with dose.
Hong (ref. 34)	Rabbits	300-600 rads of x-irradiation	SGOT activity rose promptly and markedly to a maximum at 48 hr followed by a gradual decrease to normal; the effects were dose dependent.
Cole and Peterson (ref. 35)	Rats	400-2000 rads of x-irradiation	SGOT activity rose to approximately twice normal within 6 to 12 hr after exposure to doses ranging from 800 to 2000 rads. With 400 rads the increase was 130% of normal. In all instances, the enzyme activity returned to approximately normal within 24 hr.

Table 11

ALKALINE PHOSPHATASE ACTIVITIES IN CONTROL AND IRRADIATED PRIMATES

	units/ml. serum								
	Days after Irradiation								
	Baseline	1	2	4	7	15	30	60	90
Controls	13 ± 1[b]	14 ± 4	13 ± 3	11 ± 3	17 ± 5[c]	11 ± 2	10 ± 2	14 ± 2	11 ± 2
360 rads	10 ± 4	9 ± 4	10 ± 4	10 ± 4	13 ± 8	10 ± 4	6 ± 3	8 ± 5	11 ± 5
446 rads	13 ± 3[c]	14 ± 2[c]	9 ± 2	9 ± 3	12 ± 6	9 ± 1	8 ± 1	12 ± 3	12 ± 1
538	13 ± 4	16 ± 4[c]	12 ± 3	14 ± 4	14 ± 3	12 ± 3	16 ± 10	13 ± 3	13 ± 4
624 rads									
A	11 ± 3	9 ± 3	7 ± 2	8 ± 2	9 ± 4	8 ± 5	7 ± 3	10 ± 4	9 ± 4
S	12 ± 4	9 ± 2	7 ± 2	8 ± 2	9 ± 5	8 ± 5	7 ± 3	10 ± 4	9 ± 4
N-S	8	11	5	7	6	-	-	-	-
716 rads									
A	16 ± 3[c]	16 ± 3[c]	11 ± 1	10 ± 2	14 ± 3	11 ± 3	8.4[a]	15.2[a]	12.2[a]
S[a]	12	20	11	11	17	14	8.4	15.2	12.2
N-S	18 ± 2	14 ± 2	11 ± 1	10 ± 2	13 ± 3	10 ± 2	-	-	-
802 rads									
(all N-S)	11 ± 1	11 ± 3	10 ± 3	10 ± 2	17 ± 6[b,d]	11.4[a]	-	-	-

Note: The entries in the table are the means and standard deviations of the measurements of 4 bled animals (except the survivor and nonsurvivor subdivisions of the 624- and 716-rad groups). Where no standard deviation is listed, less than three measurements were available. Normal range based on 139 nonirradiated animals, 8 ± 5 units. A = All animals. S = Survivors. N-S = Nonsurvivors.

[a] One animal.

[b] Standard deviation.

[c] .01 compared with pre-established normal range.

[d] .01 compared with preirradiation baseline.

of plasma acid and alkaline phosphate activities increased within 24 hr after irradiation. Ludewig (ref. 41) also found increased activity within 24 hr, but the activity returned to control values on the 2nd day, and then was depressed during the following 3 days. The results are shown in Table 12.

Table 12

ALKALINE PHOSPHATASE ACTIVITY IN WHOLE-BODY IRRADIATED RATS (600 rads of x-irradiation)

Days after Irradiation	Control	X-irradiation
1	42	52
2	37	38
3	38	25
4	35	20
5	43	26
10	31	29

The variability of the phosphatase response to whole-body irradiation indicates that the response is not dose dependent, and therefore phosphatases are not suitable as biological radiation dosimeters.

F. Beta-Glucuronidase

Gasso and Billiteri (ref. 42) irradiated guinea pigs with 500 rads of x-irradiation and initially reported a 33% increase in beta-glucuronidase activity 24 hr after irradiation. After 3 days the increase was approximately 35%. As shown in Table 13, these investigators (ref. 43) subsequently confirmed the increased

beta-glucuronidase activity. At 3 days after irradiation the increase in beta-glucuronidase activity was approximately 27%. Further work would be required to establish whether alterations of beta-glucuronidase activity are radiation dose dependent, as suggested by these investigators.

Table 13

BETA-GLUCURONIDASE ACTIVITY IN THE SERUM OF WHOLE-BODY X-IRRADIATED GUINEA PIGS

Radiation Dose, rads	Activity Units: Time after Irradiation 0	2 hr	5 hr	1 day	3 day	9 day
0	233	239	222	227	2[illegible]	230
500	237	332	372	282	3[illegible]	285

G. Carbonic Anhydrase

Marek and Kismider (ref. 44) reported that rabbits treated with x-ray doses of 500, 700, and 1000 rads exhibited a reduction in carbonic anhydrase activity within 24 hr after irradiation. No correlation could be established between the reduced activity and radiation dose. The enzyme activity returned to the initial or even a higher value, after approximately 48 hr. Ivanov and Dmitriev (ref. 45) exposed rabbits to a single x-ray dose of 1200 rads and determined that carbonic anhydrase activity of the blood was reduced to 80% of the initial value in the first hours after irradiation.

H. Aldolase

Aldolase activity in blood serum after whole-body irradiation varied with the type of animals employed in the experimental studies.

Dalos (ref. 46) reported that whole-body exposure of guinea pigs to 500 rads of x-irradiation resulted in an increase of serum aldolase activity from 34.1 to 64.6 units within 4 hr.

After 6 hr, the activity was 53.3 units. On the other hand, Zicha (ref. 47), working with rats, found that 24 hr after irradiation with 800 rads of x-irradiation, the mean value of serum aldolase activity decreased from 197 to 37 units. Musiiko (ref. 48) irradiated rabbits with 900 rads of x-irradiation and reported that the aldolase activity was not changed at 24 hr but was diminished after 72 hr. The observation that serum aldolase activity in irradiated rabbits is unchanged 24 hr after irradiation was also reported by Albaum (ref. 5). His results are shown in Table 14.

Table 14

SERUM ALDOLASE ACTIVITY OF IRRADIATED RABBITS
(750 rads of x-irradiation)

	Activity Units		
	Time after Irradiation, hr		
Sample	0	6	24
Control	20	79	93
Experimental	21	44	94

IV. SUMMARY

A literature review of the effects of whole-body ionizing irradiation on blood serum enzymes has been carried out to obtain information on whether alterations in the activities of specific enzymes may provide a basis for the development of a biological dosimeter. The information presented in this report discusses selected enzymes and their response to irradiation within the constraints of a practical biological radiation dosimeter for the LD_{50} range of man.

In general, enzymes appear in great quantities in the plasma when a sufficient amount of tissue is in poor physiological condition. The view is usually accepted that the enzymes diffuse abnormally from the cells into the plasma as a consequence of increased permeability, which may be considered an early, nonspecific reaction of every cell when its metabolism is grossly disturbed. Thus, altered enzymatic activities can be expected in the plasma of man and animals soon after exposure to a sufficient level of irradiation.

The usefulness of a particular enzyme system as an index of radiation exposure is frequently limited by large inherent variations in normal physiological output. Consequently, close integration of the response of the biological indicator with radiation symtomology appears necessary to ensure the validity of the biological dose-response measurement. For example, the determination of specific enzyme activity and lymphocyte counts may be of prognostic value if performed relatively soon after exposure to whole-body irradiation.

Of the enzyme systems reviewed during this study, lactic dehydrogenase isoenzymes and cholinesterase appear to hold the most promise as possible biological radiation dosimeters. Regarding LDH isoenzymes, Hawrylewicz and Blair (ref. 8, 9, 10) have shown that various levels of radiation dosage, including the LD_{50} range for man (300 to 600 rads), can be distinguished by means of the values obtained for the ratio of isoenzyme bands 1:2.

They determined that the ratio in normal rhesus monkeys 24 hr after sham exposure is within the range of 1.0 to 2.5. With 200 to 500 rads of gamma irradiation, the range decreased to 0.88 to 0.36. At levels above 500 rads serum LDH isoenzyme response was further magnified and could readily be distinguished from the responses to the lower levels of irradiation. Another favorable factor in the consideration of isoenzymes as possible biological radiation dosimeters is that the isoenzyme assay procedure has been perfected by Hawrylewicz and Blair (ref. 10) to the extent that quantitative evaluations can now be performed within 45 min.

Serum cholinesterase activity of animals exposed to whole-body ionizing irradiation generally showed a 15 to 30% decrease that persisted over a 2- to 3-day period. Although this observation was based on a relatively limited number of studies, the response appeared to be consistent and reproducible. Nevertheless, additional experimental studies, especially with primates, are required to further evaluate the reliability of employing serum cholinesterase activity as a biological radiation dosimeter.

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ABSTRACT

The purpose of this study was to review the technical literature to obtain information on whether whole-body exposure of animals to ionizing radiation in the approximate LD_{50} range for man (300 to 600 rads) induces changes in the activities of specific blood serum enzymes that are dose-related and suitable as a basis for the development of a biological radiation dosimeter. Of the enzyme systems reviewed, lactic dehydrogenase (LDH) isoenzymes and cholinesterase appear to have the most promise. Values obtained for the ratio of LDH isoenzyme bands 1:2 showed that various levels of ionizing radiation, including the LD_{50} range for man, can be distinguished. At 24 hr after sham irradiation the relative ratio of bands 1:2 of normal (control) male rhesus (Macaca mulatta) monkeys was in the range of 1.0 to 2.5, whereas at 24 hr after 200 to 500 rads of gamma irradiation of monkeys the ratios ranged from 0.88 to 0.36. At radiation levels above 500 rads, the LDH isoenzyme response was further magnified. The serum cholinesterase activities of various species of animals exposed to whole-body ionizing irradiation generally showed a 15 to 30% decrease, a response that persisted over a 2- to 3-day period. Although this observation was based on a relatively limited number of evaluations, the response was consistent. Additional experimental work, especially with primates, is required for further evaluation of cholinesterase as a biological radiation dosimeter. The usefulness of a particular enzyme system as an index of radiation exposure is frequently limited by large variations

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ABSTRACT (cont.)

inherent in normal physiological output. Consequently, close integration of the response of a biological indicator with radiation symptomology is necessary to ensure the validity of the biological dose-response measurement. For example, determination and correlation of specific serum enzyme activities and lymphocyte counts may be of prognostic value if performed soon after exposure to whole-body irradiation.

KEYWORDS: Whole-body exposure
Ionizing radiation
Blood serum
Lactic dehydrogenase isoenzymes
Cholinesterase
Rhesus monkeys, Macaca mulatta
Human LD_{50} dose range